CHEM 5181 Laboratory #1 – MALDI Time-of-Flight Mass Spectrometry

Dates: September 20 & 21, 2011
Reports Due:

Introduction:

In this lab you will analyze a mixture of three peptides using a matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometer (TOFMS) from PerSeptive Biosystems (now Applied Biosystems). You will investigate how different matrices affect the outcome of a MALDI spectrum. Then you will manipulate various parameters of the instrument to see what effect they have on the resulting spectra (e.g., ion time of flight, resolution, signal to noise).

You will be provided:

- Instrument Data Sheet and description
- Simplified instructions for using the MALDI-TOFMS
- A MALDI sample plate with four sample spots on it

  Each spot contains a mixture of three peptides,
  - Angiotensin II mH^+ = 1046.62 Da
  - Angiotensin I mH^+ = 1296.69 Da
  - Glu1 fibrinopeptide B mH^+ = 1570.68 Da

  and a different matrix:
  - α-CHCA (α-Cyano-4-hydroxycinnamic acid)
  - SA (Sinapinic acid)
  - DHB (2,5-Dihydroxybenzoic acid)
  - (no matrix)

****** NOTE – If you continually take mass spectra without moving the laser vaporization point, the sample may be used up causing loss of signal. To avoid this, you should re-aim the laser beam slightly between each run. ******

Laboratory Directions:

I. Effect of Different Matrices:

1. Using the Instrument Setting file for Linear TOFMS and the default calibration, take a spectrum for each of the four matrix sample mixtures. Start with the laser intensity set at 1500. Collect a spectrum in which the maximum signal intensity is $> 10^3$ for each mixture. If the signal is less than 10^3 increase the laser intensity until a spectrum is obtained with signal above 10^3. (Do not exceed, however, a laser intensity of 2300.)

   A. Use the S/N Calculator in the data analysis program and compare the signal to noise of all spectra that show the peptide signals.

B. Are there any additional peaks that appear in one spectra and not another?
C. Based on the above answers which matrix is the most appropriate for this mixture of peptides? Explain.

Select the most appropriate of the four sample-matrix combinations and use this spot throughout the remainder of the lab. Be sure to indicate on your lab report which spot/matrix you have chosen.

II. Effect of Instrument Calibration:

1. Using the Instrument Setting file for Linear TOFMS and the default calibration, collect a mass spectrum of the peptide mixture.

   A. What are the observed masses for the monoisotopic peaks of Angiotensin I, Angiotensin II and Glu1 fibrinopeptide B?
   B. Compare the observed masses to the expected values of the three peptides by computing the mass accuracies in ppm. (Adjust the laser intensity to ensure the detector is not saturated, i.e., signal intensity sufficiently below 6×10^4)

2. Calibrate the TOFMS using the peaks corresponding to Angiotensin I, Angiotensin II and Glu1 fibrinopeptide B *(see the MALDI Instruction packet)*. Save the calibration file (.cal) in your Data folder.

   A. What are the observed masses of the monoisotopic peaks of Angiotensin I, Angiotensin II and Glu1 fibrinopeptide B?
   B. Compute values of the mass accuracies in ppm. Are these values better than those obtained in step 1?
   C. How might you improve the calibration of the TOFMS?

III. Calculation of Mass Resolution using the computer and the Oscilloscope:

1. Use the Resolution Calculator in the data analysis program to determine the resolution for Angiotensin I, Angiotensin II and Glu1 fibrinopeptide B. *(If the resolution will not calculate (R-nc), then shoot a second sample in a different location or increase the laser intensity.)*

   Then, using the oscilloscope, determine the times of flight for the three peptides and compute the resolution directly from the observed peak widths using the equations derived in your prelab.

IV. Effect of Instrument Parameters (Use Angeotensin II for this part):

1. Vary the laser intensity from 1500 to 2000 with a step size of 100 (or 50 if necessary). Remember to move the laser target between each spectrum.

A. What changes do you see in the mass spectra (e.g., times of flight, signal intensities, resolution)?
B. Are these observations consistent with what you would expect? Explain.

2. Set the laser intensity to a fixed value to give both good signal and resolution. Hold the grid voltage constant (95%) and vary the delay time from 25 ns to 200 ns. Record and plot ion times of flight and mass resolution as a function of delay time.

   A. What is this delay? How does it apply to your sketch?
   B. Explain the shape of the graph. Do you see an optimum?

3. Hold the delay time constant at 125ns and vary the grid voltage at the following values 90, 92, 94, 95, 96, and 98%. Record and plot ion times of flight and mass resolution as a function of grid voltage.

   A. Explain the shape of the graph. Do you see an optimum?

V. Comparison of Linear and Reflectron Modes:

1. Load the Instrument Setting file for the Reflector TOFMS configuration and acquire a spectrum. Calibrate the TOF for the reflector mode and save the calibration file (.cal) in your Data folder. Record the times of flight and resolution values for Angiotensin II, Angiotensin I and Glu1 fibrinopeptide B.

   A. Referring to your sketch from the prelab, discuss the purpose of the reflector.
   B. Compare the flight times and resolutions to those obtained in the linear mode.

2. Calculate the effective flight path lengths in linear and reflector modes.

   A. How do these compare to the path lengths quoted in the manual?
   B. Explain any observed trends in the effective path lengths for all three masses. Are these trends the same for both the linear and reflector modes?
   C. How does the flight path length affect TOF mass spectra?

Log off your computer account and clean up the laboratory space before you leave.

For your lab report, please turn in answers to each of the lab questions, including all relevant graphs, calculations and discussions.